REMARKS

Applicants respectfully request the reconsideration and re-examination of the present application.

Claims 1-11 are pending in the present application. Claims 1-11 remain unchanged. As requested by the Examiner, applicants provide herewith a copy of PCT/NL92/00213, filed on November 20, 1992.

In the outstanding Official Action, claims 1-2 and 9-11 were rejected under 35 USC 102(e) as allegedly being anticipated by PALL et al. This rejection is respectfully traversed.

Applicants believe that PALL et al. fail to anticipate the claimed invention. As the Examiner is aware, a claim is anticipated only if each and every recitation as set forth in the claim is found either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. vs. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ 2d 1051, 1053 (Fed. Circ. 1987). Applicants believe that PALL et al. fail to teach a test device wherein the wells have the claimed volume.

PALL et al. disclose a device for separating plasma from blood. The device has a fibrous structure. Blood is applied at one region and the components move along the fiber due to the absorbing property of the fiber. During this process, a plasma front advances ahead of the red cell boundary. By doing so, plasma is separated.

The device according to PALL et al. is not a test device according to the present invention. This is demonstrated by column 11, lines 21 and further, which has been cited by the Patent Office.

Indeed, column 11, line 28 refers to Figure 3. Figure 3 does not show a test device comprising a well. Figure 3 shows two strips that are attached to each other.

Moreover, column 11, lines 30-35 describe that blood was placed in contact with one end of the strip and plasma was separated and collected. Even if this strip were considered to be a test device comprising a well, it would still not be a test device according to the present invention because the volume of the strip is more than 20 microliters. This is shown in column 11, lines 32-35, which states that the average volume of plasma collected was 5.7 µl with an average efficiency of 27.7%. Column 9, lines 62-62 shows how an efficiency is calculated: the volume of plasma collected is divided by the volume of blood supplied and multiplied by 100. Hence, a volume of collected plasma of 5.7 µl with an efficiency of 27.7% means that 20.6 microliter of blood was supplied (5.7/20.6 x 100 = 27.7). Thus, a strip according to PALL et al. has a volume of at least 20.6 microliter.

Moreover, a further volume has to be available in order to allow the blood components to move to a second region of the strip, during which process plasma is separated from the blood.

A strip according to PALL et al., therefore, has a significantly larger volume than 20 microliter.

Thus, PALL et al. fails to anticipate the claimed invention.

Claims 3-8 were rejected under 35 USC 103(a) as allegedly being unpatentable over PALL et al. This rejection is respectfully traversed.

Applicants respectfully submit that PALL et al. also fail to render obvious claims 3-8. While the Office Action contends that it would have been obvious for one of ordinary skill in the art to modify PALL et al. to have a well density of 10 to 15 wells per square centimeter, applicants do not believe that the densities of the wells would be a parameter that would be readily optimized by one of ordinary skill in the art. Indeed, as the Examiner is aware, a particular parameter or variable must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the parameter or variable might be characterized as routine or obvious. *In re Antonie*, 559 F.2d 618, 195 USPQ 6 (CCPA 1977). See also *In re Boesch*, 617 F.2d 272, 205 USPQ 215 (CCPA 1980).

As already acknowledged by the Patent Office, PALL et al. are completely silent as to the claimed depth, the diameter ratio of the wells and the density of the plate. As a result,

applicants believe that it cannot be said that PALL et al. characterize these parameters as routine or obvious.

The Examiner is also respectfully reminded that a critical step in analyzing obviousness pursuant to 35 U.S.C. §103(a) is casting the mind back to the time of the invention, to consider the thinking of one of ordinary skill in the art, only guided by the publications and then-accepted wisdom in the field. Close adherence to this methodology is important in cases where the invention itself may prompt an Examiner to "fall victim to the insidious effect of a hindsight syndrome, wherein that which only the invention taught is used against its teacher." Indeed, to establish a prima facie case of obviousness, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. *In re Kotzab,* 217 F.3d 1365, 1369-70, 55 USPQ 2d 1313, 1362 (Fed. Circ. 2000). The fact that the prior art could be so modified would not have made the modification itself obvious unless the cited publications themselves suggested the desirability of the modification. *In re Gordon,* 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Circ. 1984).

In light of the lack of a motivation, suggestion or teaching of the desirability of making the claimed combination, applicant believes that the publications fail to disclose or suggest the claimed invention.

At this time, applicants note that the Office Action states that applicants believe that the priority date of the presently-claimed subject matter goes back to the PCT application filed on November 20, 1992. Furthermore, the Office Action states that the Office will accept this statement as fact. In doing so, the Office Action suggested that applicants provide a copy of the earliest document pointing out where the support for the claimed invention may be found.

As requested by the Examiner, applicants submit a copy of the PCT/NL92/00213 application, filed on November 20, 1992. While this is not the earliest application (the present application also claims priority to the Netherlands application No. 9101953, filed on November 21, 1991), applicants note that the filing date of the PCT application is earlier than the references cited in the previous Office Action.

Applicants would also like to note that in the amendment filed on January 3, 2005, applicants only pointed out that the Patent Office had failed to satisfy its burden in showing that the STEDRONSKY patent qualified as prior art. Additionally, applicants noted that STEDRONSKY, like BERENSON et al., failed to teach the claimed volume, depth and diameter of the wells. As a result, applicants believe that the Patent Office also failed to meet its burden in showing that even if STEDRONSKY qualified as prior art, STEDRONSKY did not disclose nor suggest the claimed invention.

Indeed, as noted in the amendment of January 3, 2005, the designation of an application as a continuation-in-part (CIP) does not, by itself, have any legal effect whatsoever on the question of whether the clams in CIP are entitled to the filing date of a parent application. Claims that are fully supported by the disclosure found in the parent application are entitled to the benefit of the filing date from that application. Claims which depend upon the new disclosure of the CIP application for enablement, a written description and/or best mode, are not entitled to the benefit of the filing date from the parent application. Claims of the parent case that are carried over into the CIP application or are new will be entitled to the filing date of the parent application as long as the parent application contains sufficient disclosure to support the claims.

In view of the above, applicants believe that the present application is in condition for allowance at the time of the next Official Action. Allowance and passage to issue on that basis is respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any

overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX

The Appendix consists of a copy of the PCT/NL92/00213 application, filed on November 20, 1992.

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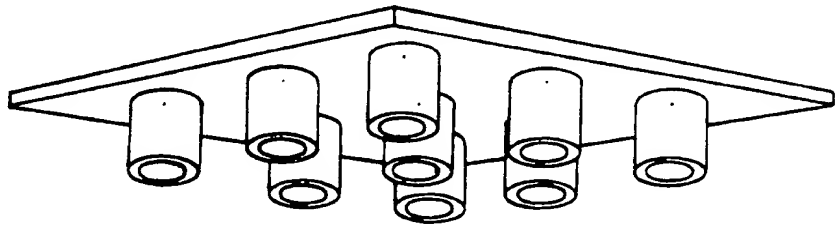
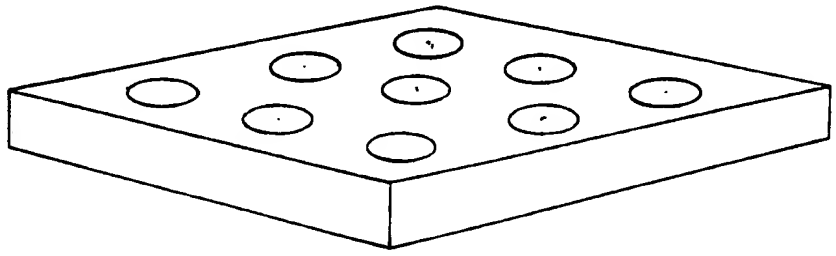




(57) Abstract

The invention relates to a test device comprising a plate containing a multiplicity of wells. The invention also relates to a metering device suitable for simultaneously introducing equal volumes of reagent into different wells of the test device. The test device comprises wells which have a volume within the range of 0.1-20 µl. The ratio between the depth of the wells and the diameter is preferably less than 1:1. The test device according to the invention can be used for carrying out a bio- or immuno-chemical test such as a pepscan or ELISA.

Test device comprising a plate containing a multiplicity of wells with an associated metering device, as well as a kit which comprises these devices and use of the devices.

The present invention relates to a test device comprising a plate containing a multiplicity of wells. The invention also relates to a metering device suitable for the simultaneous introduction of equal volumes of reagent into different wells of the test device. In addition, the invention relates to a method for carrying out a (bio- and/or immuno) chemical test using the test device and/or the metering device and the invention is aimed at a kit which comprises the test device and the metering device.

A test device comprising a plate containing a multiplicity of wells has already been known for years in the form of the so-called microtitre plate. The known microtitre plate is of the order of 12.5 cm × 8.0 cm in size and comprises 96 wells. The diameter of each well is about 0.6 cm and the depth of each well is about 1.0 cm, so that each well can contain at most a 250 µl sample. The wells are separated by material barriers with a width of approximately 2.0 mm.

The known microtitre plate is used when carrying out diverse bio- and/or immunochemical tests. In tests of this type, photometric detection is frequently used. A very well-known example of such a test is the ELISA. In the case of photometric determinations, the bottom of a well must be uniformly covered with a layer of the sample to be analysed in order to obtain reliable results. Furthermore, this layer must have a thickness which is at least such that detectable absorption occurs. In practice, this generally implies that samples are used which have at least a volume of 50 µl.

Since bio- and/or immunochemical tests frequently involve large numbers of tests on samples (such as blood and sera) which have to be obtained from test persons and/or animals, there is a need to use as little sample as possible per test.

However, the sample can be diluted to only a limited degree since the component to be analysed must also be present in the well in a certain minimum concentration in order to obtain measurable absorption. This is because, according to Lambert-Beers' law, the light intensity is dependent on the concentration and the absorption coefficient of the component to be analysed and also on the distance the light has to travel through the sample to be measured. In practice, this implies the use of about 12.5 ml

of reagents per microtitre plate. In such cases there is an appreciable need to reduce the amount of sample to be used.

Another frequent use of the current microtitre plate is in the synthesis of peptides. In such syntheses peptides containing different amino acid sequences can be synthesised. This can be carried out, for example, with a view to determining the location of an epitope of a protein for a specific antibody. To this end, peptides containing amino acid sequences corresponding to a fragment of the protein to be studied are synthesised separately. The synthesis can be carried out in such a way that each peptide in part contains the amino acid sequence of another peptide. It is even possible to carry out the synthesis in such a way that only one amino acid does not overlap. It is also possible to produce a series of short sections, for example hexapeptides which overlap with the exception of one amino acid. A determination is then carried out to establish with which peptide antibody binding takes place. Peptides with which antibody binding takes place contain an epitope.

In the first instance, peptide synthesis was carried out by adding the amino acid to be coupled to the well of the known microtitre plate in which the peptide had to be synthesised, then coupling the desired amino acid to the growing peptide chain, subsequently washing the well to remove any unreacted amino acid and repeating the procedure with the next amino acid.

However, with this method problems were experienced in rinsing the wells and, therefore, a method of peptide synthesis was adopted in which small polyethylene rods are used as supports for the growing peptide chains. This method is described by Geysen, H.M., Meloen, R.H. and Barteling, S.J. beschreven in Proc. Natl. Acad. Sci. USA, Vol. 81 (July 1984) pp. 3998-4002. In this article, a method is described for the simultaneous synthesis of hundreds of peptides on a solid support with adequate purity for carrying out an ELISA. Interaction of the peptides with antibodies can be detected simply, without removing the peptides from the support. Consequently, it becomes possible to determine an immunogenic epitope with a good resolution. This method is termed the PEPSCAN.

With this method, the growing peptide chains are allowed to adhere to polyethylene rods (having a diameter of 4 mm and a length of 40 mm) and the reactions required for peptide synthesis are then carried out using the ends of the support rods. To this end, the polyethylene rods are first immersed in a 6 percent solution of acrylic acid in water and subjected to γ-radiation. For the subsequent reactions, the ends of the rods are then

brought into contact with a Teflon plate containing a matrix of wells corresponding to the location of the rods (the known microtitre plate). The conventional methods for peptide chemistry in the solid phase can be used here, for example for coupling N^{α}-t-butyloxycarbonyl-L-lysine methyl ester to polyethylene/polyacrylic acid via the N^{α}-amino group of the side chain. [(Erickson, B.W. and Merrifield, R.B. (1976) in The Proteins, Eds. Neurath, H & Hill, R.L. (Academic, New York), Vol. 2, pp. 255-527) and (Meienhofer, J. (1973) in Hormonal Proteins and Peptides, Ed. Li, C.H., (Academic, New York), Vol. 2, pp. 45-267)]. After removing the t-butyloxycarbonyl group, t-butyloxycarbonyl-L-alanine can be coupled, a peptide-like spacer being formed. The desired amino acids can be coupled successively and, following the final desired coupling reaction and after removal of the protecting t-butyloxycarbonyl group, the terminal amino acid can be acetylated using acetic anhydride in dimethylformamide/triethylamine. All coupling reactions carried out with N,N-dicyclohexylcarbodiimide can be carried out in dimethylformamide in the presence of N-hydroxybenzotriazole. Any protective groups in side chains of amino acids used in the peptide synthesis can also be removed. Before the synthesised peptides are examined further, for example by means of ELISA, the rods can be washed with a phosphate-buffered saline solution.

Another use of peptide synthesis takes place if one or more amino acids of a known epitope are changed in order to determine which other sequences are able to function as an epitope and/or in order to determine which amino acids are essential for the epitope action. A method of this type is described by Geysen, H.M., Meloen, R.H. and Barteling, S.J. in Proc. Natl. Acad. Sci. USA, Vol. 82 (January 1985) pp. 178-182.

As such methods frequently comprise a large number of syntheses and thus also the use of large amounts of reagents, which reagents, moreover, are frequently expensive, there is also a need, with a view to reducing costs, to use sample amounts which are as small as possible. Possibilities have therefore been sought for miniaturisation of such peptide syntheses.

A method for miniaturised peptide synthesis has recently been described in an article by Fodor, S.P.A. et al. (Science, (15 February 1991) pp. 767-773). In this method light is used to control the simultaneous synthesis of a large number of different chemical compounds. Synthesis takes place on a solid support, such as a glass plate. The support is aminated by treatment with 0.1 % aminopropyltriethoxysilane in 95 % ethanol. Here, a light-sensitive protective group is then introduced, said protective group disappearing following irradiation with light and giving

a reactive site to which a building block, such as an amino acid, can be coupled. The pattern in which exposure to light or other forms of energy takes place (for example via a mask) determines which areas of the support are activated for chemical coupling. The entire surface is brought into contact with the building block to be coupled (said building block also being provided with a light-sensitive protective group). A coupling reaction will occur only at sites where the light in the previous step has given rise to activation. The substrate is then exposed through another mask, so that a subsequent building block can be incorporated in the desired site. The pattern of the mask and the sequence of the reagents determine the sequences of the peptides formed. A high degree of miniaturisation can be achieved in this way. For example, it is possible to synthesise 40,000 different peptides on 1 cm^2.

However, this method has a number of disadvantages. The removal of the protective light-sensitive group (nitroveratryloxycarbonyl is named in the article) takes place by irradiation for 20 minutes with a mercury lamp having a power of 12 mW/cm^3. This will result in a very long synthesis time in the case of the synthesis of longer peptides. Furthermore, a different mask will have to be used for each addition step and a different set of masks will have to be used for each series of peptides.

Moreover, only one building block can be added in each addition step because the various peptides to be synthesised are not spatially separated. It is obvious that mixing of reagents would otherwise take place and, thus, undesired products would also form. This method is therefore very laborious, especially for the synthesis of peptides which differ not only in respect of length but also in respect of sequence.

The authors of the article themselves also touch on the problem of the reliability of the synthesis. Deletions can occur as a consequence of incomplete removal of the protective group, following irradiation with light. The net coupling percentage is 85-95 %. Furthermore, when changing masks, a certain overlap between the diverse synthesis regions will take place because of light diffraction, internal reflection and scattering. Consequently, compounds will be formed in regions which are considered to be dark, as a result of which undesired insertion of a specific amino acid can take place.

The present invention relates to a test device which solves the miniaturisation problems described above and is suitable for use for bio- and/or immunochemical tests such as ELISA and tests in which peptide syntheses are used, for example the PEPSCAN as described above.

The present invention relates to a test device which comprises a plate containing a multiplicity of wells, which is characterised in that the wells have a volume within the range of 0.1-20 µl. The dimensions of the wells will be chosen depending on the price and availability of the samples and reagents to be used. In general, wells which are as small as possible will be preferred and, therefore, the present invention preferably relates to a test device in which the wells have a volume within the range of 0.1-5 µl.

Entirely contrary to expectations, it has now been found that making the wells smaller has no adverse consequences for the efficient rinsability thereof. It has been found that the rinsing times required in order to obtain good rinsing are shortest if the ratio between the depth of the wells and the diameter thereof is less than 1:1. Therefore, a test device comprising a plate containing a multiplicity of wells, characterised in that the wells have a volume within the range of 0.1-20 µl, and that the ratio between the depth of the wells and the diameter thereof is less than 1:1, is very suitable. A test device according to the invention for which the ratio between the depth of the wells and the diameter thereof is less than 2:3 is preferred.

Figures 1 and 2 show the results of tests in which the rinsability of various test devices was investigated. The test devices had wells of equal diameter (2 mm) but of different depths. The rinsability was investigated on a shaking machine at speeds of, 47 and 40, respectively. The depth of the well in mm is plotted against the time in minutes needed to properly rinse the well.

The invention is preferably aimed at a test device in which the wells have a diameter of 1.0-4.0 mm, a diameter of 1.0-2.0 mm being preferred. The choice of the dimensions of the wells will depend on the desired specific test for which the test device is to be used. The smaller the diameter, the smaller the required volume of the sample.

In connection with the desired good rinsability, it is also preferred that the wells have a shape such that a vertical cross section of the wells is essentially U-shaped, the transition between legs and base of the U being gradual. Preferably there are no sharp angles in the well.

A number of suitable shapes of wells are shown in Figure 3.

The U shape in which the angle between base and legs is perpendicular is preferred for photometric determinations in which measurement is carried out under and through the plate.

The test device according to the invention will preferably be a plate containing wells separated by material barriers with a width of 1.0-5.0 mm, preferably by material barriers with a width between 1.0 and 2.0 mm. The material barriers must be sufficiently wide to prevent reagents flowing over from one well to another. Specifically, the material barriers must be sufficiently wide when DMF is used as solvent, as is frequently the case in peptide synthesis. This is because DMF is known to have a high creeping capacity.

The test device according to the invention will comprise a plate containing 5-20 wells per cm^2, preferably 10-15 wells per cm^2.

Furthermore, the test device will comprise material to which peptides, proteins and other biochemical molecules, such as hormones and polysaccharides, are able to adhere. For test devices suitable for tests with peptide synthesis, such material will preferably be material to which peptides and proteins are able to adhere, such as polyethylene or polystyrene.

Other suitable examples of materials which can be used in a test device according to the invention are polypropylene and polycarbonate.

The choice of the material for the test device will also depend on the reagents to be used in such a test device and on the detection method. In the case of photometric analysis through the bottom of the test device, for example, at least the bottom of the wells will have to be composed of transparent material. In the case of tests where DMF is used as solvent, it will not be possible to use a polystyrene test device because DMF is too aggressive.

A preferred embodiment of the test device will be provided with a means for recording information, for example a bar code or a magnetic strip. The test device can be provided with data relating to the test it is intended to carry out, or which has been carried out, such data for example relating to reagents or samples which have been used. The test device can also be provided with markings which indicate the coordinations of wells in the test device.

Figure 4 shows a top view of an example of one embodiment of the test device according to the invention.

A cross section is shown in Figure 5.

As already mentioned in the preamble, the present invention is also aimed at a method for carrying out (bio- and/or immuno)chemical tests, in which a test device according to the invention is used. In general, a test device according to the invention can be used in the same tests as the

known microtitre plate. It is now possible to carry out existing methods using much smaller amounts of sample and reagents; so-called mini-tests are now possible. It is possible to reduce the amount of sample used by a factor of one hundred. It is now possible to use 2.5 µl instead of 250 µl samples per well. Use of the test device according to the invention now makes mass screening of population groups much more attractive because much less blood is required from the donor and much smaller quantities of reagents are required.

Another great advantage of the miniaturisation of the methods by use of the test device according to the invention lies in the fact that the existing chemistry does not have to be modified. In this context consider the great advantage, for example, in the case of automated processes, such as the PEPSCAN. The test device is particularly suitable for use in methods in which large numbers of samples have to be used. A mini-ELISA and mini-PEPSCAN in which a test device according to the invention is used are suitable examples of the method according to the present invention. The advantage of a mini-method according to the invention is that the test can be carried out with sample amounts of less than 20 µl. It is readily possible to use sample amounts of less than 5 µl in a mini-method according to the invention.

The present invention is also aimed at a metering device suitable for simultaneously introducing equal volumes of reagent into different wells in a test device according to the invention. A metering device according to the invention can be used in order to carry out as efficiently as possible immuno- and/or biochemical tests in which a test device according to the invention is used. In this context, consideration is given to optional automation of certain methods according to the invention.

If a predetermined equal amount of reagent has to be introduced simultaneously into a number of wells, it is possible to use a metering device according to the invention, said device being provided with projections having dimensions and mutual spacings such that individual projections can simultaneously be positioned in or above wells of a test device according to the invention. With a metering device of this type, all wells can simultaneously be provided with equal volumes of reagent if the position of the projections is such that this essentially corresponds to the position of the wells in the test device according to the invention.

One embodiment of the metering device and the test device is shown in Figure 6.

If not all, but only a certain number, of wells in the test device have to be filled, a metering device can be used which has projections which simultaneously can be positioned above or in the selected wells.

In Figure 7 the darker wells are the selected wells. The projections of the metering device are located on the metering device in such a way that they can simultaneously be positioned above or in the darker wells.

To this end, a metering device can advantageously be used in which the projections are fixed to the support or can be fixed in a pattern which corresponds to the pattern of the wells into which reagent has to be introduced.

Figure 8 shows an example of an embodiment of the metering device in which the projections can be fixed to a support.

The metering device can comprise projections which are fixed or can be fixed to a support in a way equivalent to the bristles of a brush (Figs. 6, 7 and 8).

A metering device in which the projections, like the teeth of a comb, are parallel to one another and are fixed (Fig. 9) or can be fixed (Fig. 10) at their tops to a support is also an embodiment of a metering device according to the invention which is very suitable. The number of projections can be less than or equal to the number of wells forming a row in the longitudinal direction of the test device. The number of projections can be less than or equal to the number of wells forming a row in the widthwise direction of the test device. The number of projections will depend on the pattern of wells of the test device into which reagent has to be introduced.

The projections of a metering device according to the invention can be integral with the support or can be detachable. The projections can be fitted on the support in such a way that the projections form a pattern which corresponds to the pattern of wells which have to be filled in the test device (see Fig. 7).

It is also possible to use a metering device in which more than one projection can be positioned above or in a well at the same time, if it is desired to simultaneously introduce more than one reagent unit, which is present on a projection, into a well.

Figure 11 shows a metering device in which two projections can be positioned simultaneously above or in each well.

Thus, the amount of reagent which is present on a projection can be taken as standard and metering devices can be used which have a group of projections above or in the well, depending on the ratio in which it is

desired to introduce reagents into a well. A group will comprise the number of projections which corresponds to the number of desired reagent units.

In the case of a metering device according to the invention, the projections can be hollow, but they can also be solid or closed at the bottom. The latter two possibilities are to be preferred when working with very small amounts of sample and reagents, because it is then possible to work with drops of reagent.

The present invention is also aimed at a method for carrying out a (bio- and/or immuno-)chemical test using a metering device according to the invention, in which method

a) the projections of the metering device are provided with reagent, in such a way that essentially equal volumes of reagent are present on or in the individual projections of the metering device, and

b) the metering device is then positioned in or above wells of the test device according to the invention, which wells are intended to be provided with reagent, each individual projection being located in or above a well at the same time, and

c) essentially equal volumes of reagent are introduced into the individual wells of the test device, which wells it is intended to provide with reagent.

The invention also relates to a method of this type in which the projections of the metering device are simultaneously provided with reagent by immersing the projections in reagent.

The present invention is also aimed at a kit which comprises at least a test device and metering device according to the invention. Such a kit can comprise a number of metering devices in the various embodiments described above and can also comprise replaceable projections for such metering devices.

Example 1

Miniaturised peptide synthesis

Miniaturised synthesis of a complete tripeptide net (8000 different peptides) was carried out using test devices according to the invention. The test device used in this example resembled a credit card in size and was constructed so that it comprised 455 wells with a diameter of 2 mm and a maximum volume of 5 µl each. The test device was made of polyethylene. In order to make this solid carrier suitable for peptide synthesis the wells were treated via the method described by Geysen et al. (1984) previously mentioned. The carboxyl groups of the polyacrylic acid were provided with a NH_2-group via a linking reaction of a linker t-ButylOxyCarbonyl-HexaMethyleneDiAmine (BOC-HMDA) in the presence of N,N-DicyclohexylCarbodiimide and N-HydrOxyBenzotriazole (DDC/HOBt). All these linking reactions were carried out in DiMethylFormamide (DMF).

After removing the t-butyloxycarbonyl group with TriFluor Acetic acid (TFA) a mixture of all twenty L-amino acids linked using the same method as used for linking the linker was used.

The total volume used in the linking reaction amounted to 3 µl for each well. Pipetting the required small amounts was achieved completely automatically using a computer directed robot arm with a pipette installation (Hamilton MicroLab 2200). A special software program was written for this objective enabling two of test devices according to the invention described in this example to be filled per hour.

The linking time for each amino acid amounted to approximately 2 to 3 hours. The difference in linking time is caused by the reaction stopping when the reaction mixture has completely evaporated.

Subsequently after removal of the BOC-group with TFA the next amino acid was linked in the same manner after which the cycle was repeated twice more.

After the last linkage reaction and after removal of the BOC-group the terminal NH_2-group was acetylated with a mixture of acetic acid anhydride in DMF and TriEthylAmine in the ratio 2/5/1. The groups protecting the side groups were removed in a strong acid environment. In this instance BoriumTrisTrifluor acetic acid (BTT) in TFA (30 mg/ml) was used for two hours at room temperature.

The total structure of the peptide was as follows:

$$Ac\text{-}A_3\text{-}A_2\text{-}A_1\text{-}X\text{-carrier},$$

wherein Ac represents an acetyl group, A_x represents a single amino acid and X represents the mixture of amino acids.

Example 2

Elisa

The test devices according to the invention were rinsed with phosphate buffered saline (PBS, 3x10 min) before incubation of the peptides with serum, after which the test devices according to the invention were precoated for 1 hour at 37°C with 10% horse serum/10% ovalbumine/1% Tween 80 in PBS (SuperQ) in order to prevent aspecific absorption of antibodies. The test devices according to the invention were completely submerged in the liquid.

Filling the test device wells with serum dilution can be carried out in two ways. If only a little serum is available the test devices can be filled using the robot arm mentioned in the previous example and when sufficient serum is available the test devices can be submerged in the serum and subsequently be wiped so that all the wells are simultaneously filled.

Incubation of the test devices took place during the night at 4°C in air saturated with water, after which the test devices were washed three times with 0.05% Tween 80/PBS in order to remove antibodies that had not been bound. The test devices according to the invention that had been incubated with serum were subsequently incubated for 1 hour at 37°C with an antibody conjugated peroxidase enzyme (1/1000 solution in SuperQ) by submerging the test devices in a solution comprising said enzyme. After this the test devices were rinsed with PBS, 3x 10 min. The presence of the second antibody was demonstrated with the substrate liquid ABTS (2,2'-Azine-di[3-ethyl benzthiazoline sulfonate (6)]). In this instance it is also possible to use the two aforementioned methods to fill the wells, either using the robot arm or submerging the devices in the substrate.

CLAIMS

1. Test device comprising a plate containing a multiplicity of wells, characterised in that the wells have a volume within the range of 0.1-20 µl.

2. Test device according to Claim 1, characterised in that the wells have a volume within the range of 0.1-5 µl.

3. Test device according to Claim 1 or 2, characterised in that the ratio between the depth of the wells and the diameter thereof is less than 1:1, preferably less than 2:3.

4. Test device according to any of the preceding Claims, characterised in that a vertical cross section of the wells is essentially U-shaped, the transition between legs and base of the U being gradual.

5. Test device according to any of the preceding claims, characterised in that the diameter of the wells is 1.0-4.0 mm and preferably 1.0-2.0 mm.

6. Test device according to any of the preceding claims, characterised in that the wells are separated by material barriers with a width between 1.0-5.0 mm and preferably between 1.0 and 2.0 mm.

7. Test device according to any of the preceding claims, characterised in that the plate contains 5-20 wells per cm^2, preferably 10-15 wells per cm^2.

8. Test device according to any of the preceding claims, characterised in that the wells of the plate are composed of material to which peptides, proteins and other biochemical molecules, such as hormones and polysaccharides, are able to adhere.

9. Test device according to any of the preceding claims, characterised in that the bottom of the wells is sufficiently transparent for carrying out photometric analysis.

10. Test device according to any of the preceding claims, characterised in that the plate is made from material to which proteins and/or peptides are able to adhere.

11. Test device according to any of the preceding claims, characterised in that the plate is made from material selected from the group comprising polyethylene, polypropylene, polystyrene and polycarbonate.

12. Method for carrying out a (bio- and/or immuno)chemical test, characterised in that a test device according to any of Claims 1-11 is used.

13. Method according to Claim 12, characterised in that the test carried out is a pepscan or ELISA.

14. Method according to Claim 12 or 13, characterised in that the test is carried out using sample amounts of less than 20 µl.

15. Method according to any of Claims 12-14, characterised in that the test is carried out using sample amounts of between 0.1 and 5 µl.

16. Use of a test device according to any of Claims 1-11 for carrying out a bio- or immunochemical test such as a pepscan or ELISA.

17. Metering device suitable for simultaneously introducing equal volumes of reagent into different wells of a test device according to any of Claims 1-11.

18. Metering device according to Claim 17, characterised in that said device is provided with projections which have dimensions and mutual spacings such that individual projections can simultaneously be placed in or above the wells of a test device according to any of Claims 1-11, intended to be provided with reagent.

19. Metering device according to Claim 17 or 18, characterised in that the projections, like the teeth of a comb, are parallel to one another and are fixed at their tops to a support.

20. Metering device according to Claim 17 or 18, characterised in that the position of the projections is such that said position essentially corresponds to the position of the wells in the test device according to any of Claims 1-11.

21. Metering device according to any of Claims 17-20, characterised in that said device is provided with projections which have dimensions and mutual spacings such that, in each case, a group of projections is located above a well.

22. Metering device according to any of Claims 17-21, characterised in that the projections are closed at the bottom.

23. Metering device according to any of Claims 17-22, characterised in that the projections are detachable.

24. Method for carrying out a (bio- and/or immuno)chemical test using a metering device according to any of Claims 17-23, in which method

a) the projections of the metering device are provided with reagent, in such a way that essentially equal volumes of reagent are present on or in the individual projections of the metering device, and

b) the metering device is then positioned in or above the wells of the test device according to any of Claims 1-11, said wells to be provided with reagent, each individual projection or each group of projections being located in or above a well at the same time, and

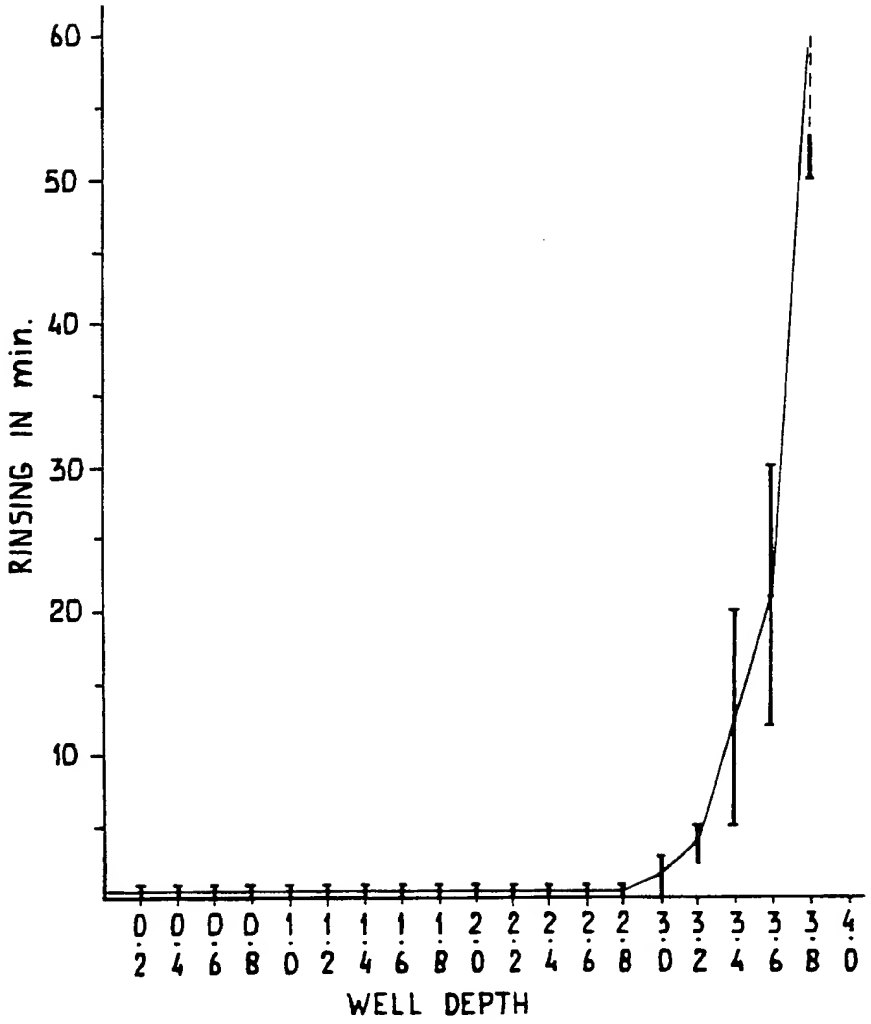
c) essentially equal volumes of reagent are introduced into the individual wells of the test device, said wells to be provided with reagent.

25. Method for carrying out a (bio- and/or immuno)chemical test according to Claim 24, in which method, in step a), the projections of the metering device are simultaneously provided with reagent by immersing the projections in reagent.

26. Kit which comprises at least a test device according to any of Claims 1-11 and at least a metering device according to any of Claims 17-23.

27. Kit according to Claim 26, which comprises detachable projections for the metering device.

Fig - 1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0.6
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1.2
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1.6
1.8
2.0
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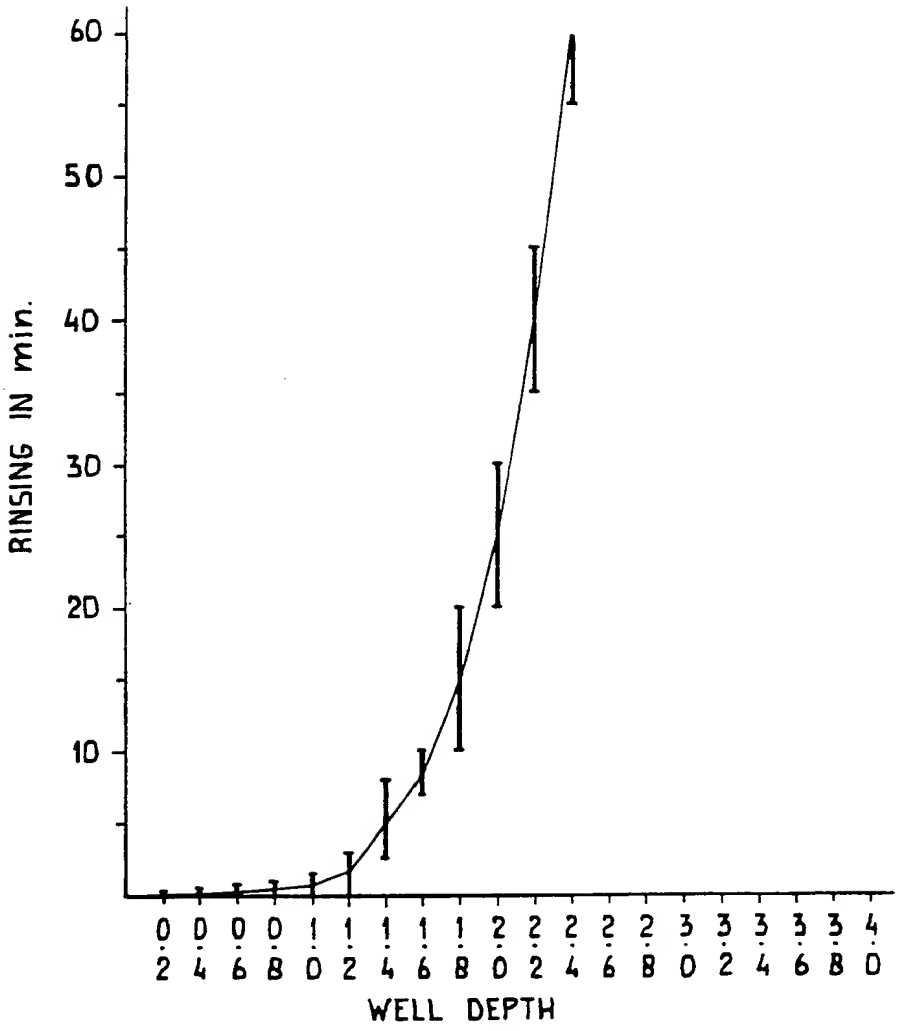
3.8
4.0
WELL DEPTH

Fig-2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RINSING IN min.
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1.8
2.0
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WELL DEPTH

fig-3

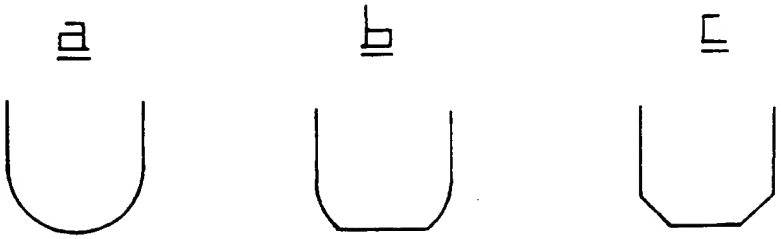


fig-4

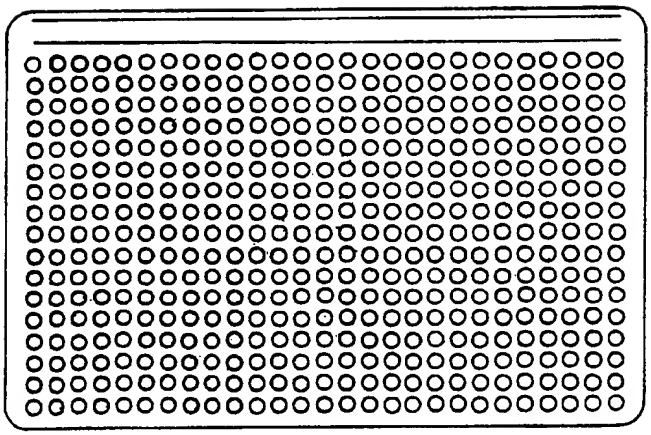


fig-5

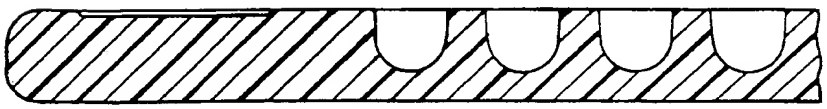


Fig-6

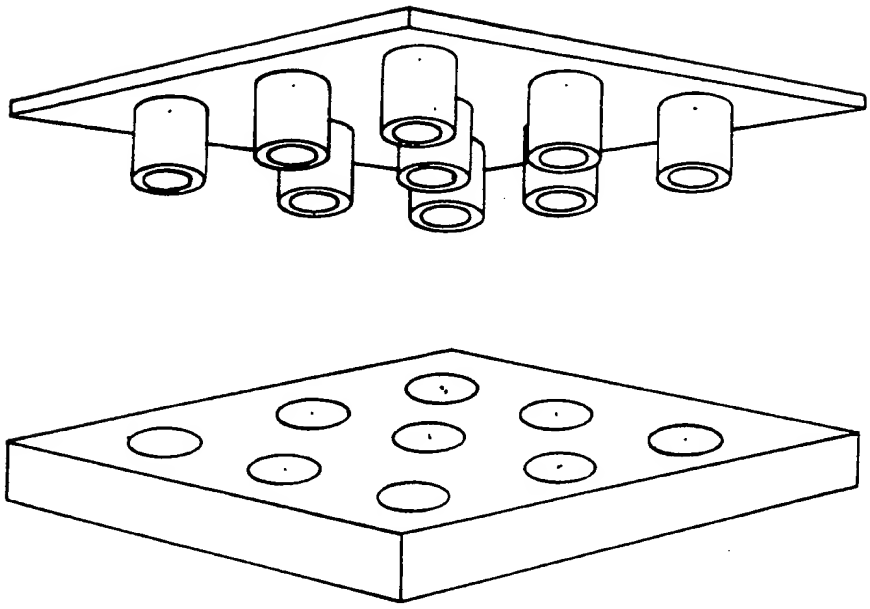


Fig-7

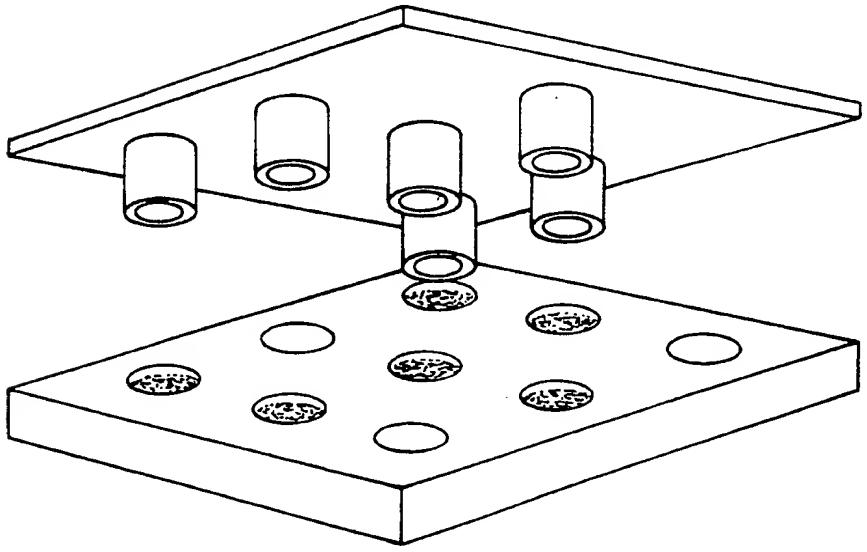


fig-8

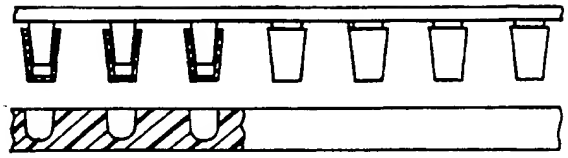


fig-9

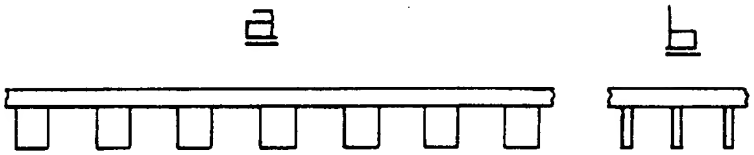


fig-10

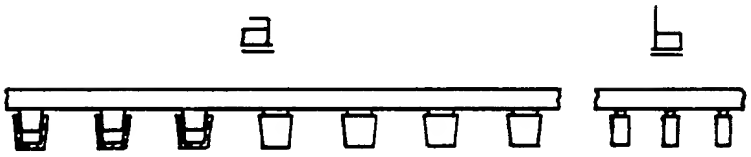
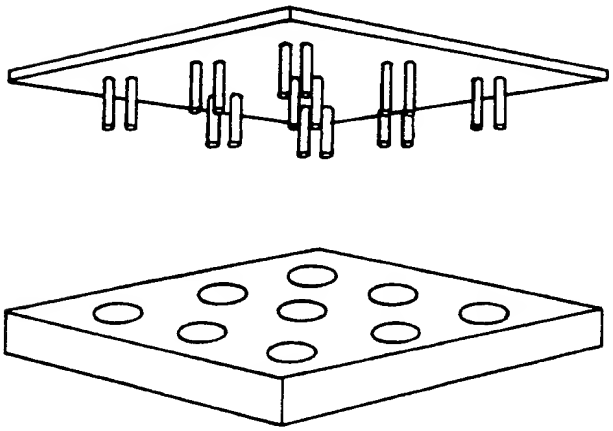


fig-11



I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 B01L3/00; G01N33/53

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	B01L

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	WO,A,9 106 859 (R.M. JAFFE) 16 May 1991 see page 16, line 6 - page 17, line 19	1,3, 8-14,16
A	EP,A,0 018 435 (W. STÖCKER) 12 November 1980 see page 4, line 7 - line 13 see page 13, line 10 - line 13	1,7,9,12
A	FR,A,2 383 442 (INSTITUT PASTEUR) 6 October 1978 see page 2, line 11 - line 33; figures 6-10	1,2, 12-27
A	DD,A,107 783 (A. HORN ET AL.) 12 August 1974 see page 4	1,12-17

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° Special categories of cited documents :[10]

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 MARCH 1993	08. 04. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	HODSON C.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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A	US,A,4 735 778 (N. MARUYAMA ET AL.) 5 April 1988	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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